REMARKS

As set forth in the application and emphasized in previous responses to Office Actions, applicants' invention is the provision of conditions under which the most primitive of hematopoietic stem cells -- cells which have the potential for differentiating into any type of hematopoietic cell -- can grow, divide and be efficiently transformed with a heterologous gene, without a concomitant loss of their pluripotent ability. This is vitally important in a successful gene therapy regimen targeting diseases that involve hematopoietic cells, and in particular such diseases that require ablation and repopulation of a patient's hematopoietic system. An alternate aspect of the invention is the synergistic effect of an mpl ligand, a flt3 ligand and fibronectin in the successful transduction with and long-term expression of a heterologous gene that is transduced into a $CD34^+$ human hematopoietic cell. As discussed in more detail below, none of the cited references teach or suggest the conditions discovered and currently claimed by applicants.

I. Claim Amendments

Applicants have amended the claims to better define their invention as described above.

Claims 18 and 23 have been amended to recite "a method for obtaining genetically modified human pluripotent hematopoietic stem cells." This makes it clear that the invention achieves the transduction of stem cells which retain the potential for differentiating into any type of hematopoietic cell. Consistent with the purpose of the invention, claims 18 and 23 have also been amended to recite that the population of cells which are to be contacted with the vector carrying the heterologous gene are "human $CD34^+$ hematopoietic cells" which include "a subpopulation of pluripotent $CD34^+Thy\text{-}1^+Lin^-$ cells." It is the $CD34^+Thy\text{-}1^+Lin^-$ cells that include the desired pluripotent stem cells. Support for this amendment is found throughout the specification and in particular at page 4, lines 4-10; page 27, lines 4-10, and in the Examples.

Claims 19-20 and 24-27 have been amended to specify that the additional cytokine recited is added to the cell culture prior to transduction. Those claims have also been amended to be consistent with amended claims 18 and 23. Support for this amendment is found in Examples 8 and 9.

Claim 37 has been amended to specify that the population of human $CD34^+$ hematopoietic cells being transduced includes a subpopulation of "pluripotent $CD34^+Thy\text{-}1^+Lin^-$" hematopoietic cells. Again, this clarifies that the invention provides methods for obtaining transformed human hematopoietic cells which retain their pluripotent ability. Support for this amendment is found throughout the specification and in particular at page 4, lines 4-10; page 27, lines 4-10, and in the Examples.

These amendments render claims 44 and 51 redundant. Accordingly, those two claims have been cancelled.

Applicants have also added claim 52 which is directed to a method of obtaining a genetically modified human hematopoietic cell by contacting a population of $CD34^+$ human hematopoietic cells cultured in the presence of fibronectin, a mpl ligand and a Flt3 ligand and optionally in the presence of one or more cytokines selected from c-kit ligand, IL-3, IL-6, and LIF, with a vector encoding a heterologous gene; and obtaining said genetically modified human hematopoietic cell. This claims embodies the synergistic effect of fibronectin, a mpl ligand and a flt3 ligand on the successful transduction with and expression of a heterologous gene in a $CD34^+$ human hematopoietic cell, which applicants have demonstrated. Support for this added claim is found in Examples 8 and 9 of the specification.

II. Claim Rejections

Claims 18-20, 23-27, 31-35, 37-44 and 46-51 stand rejected under 35 U.S.C. §103(a) as being "unpatentable" over a combination of numerous references. The Examiner is of the opinion that several of the cited references teach the combined use of an mpl ligand (TPO) and a flt3 ligand (FL) for the growth of human stem cells in culture -- the minimal cytokine combination required in applicants' claimed methods for obtaining transformed human pluripotent hematopoietic stem cells. Applicants believe the amendments presented herein obviate this rejection. None of the references taken individually or in combination provide the requisite motivation and a reasonable likelihood of success in using these two cytokines, alone or in combination with other cytokines recited in the claims, to grow and transform true pluripotent human hematopoietic stem cells without a loss of some or all of that pluripotent property.

The Examiner has cited Ku et al., Kobayshi et al., Ohmizono et al. and Ramsfjell et al. as teaching the use of a combination of TPO and FL in the growth of $CD34^+$ hematopoietic cells.

With respect to Ku et al., applicants respectfully submit that the Examiner has misinterpreted the reference. Ku et al. relates to experiments using the soluble portion of the TPO receptor (sTPOR) in combination with either a Flt3 ligand or steel factor (SF). The sTPOR used by Ku et al. is *not* a mpl ligand, as that term is defined in the present application. Applicants define an mpl ligand as "any compound capable of binding to the mpl ... receptor" (page 10, lines 16-20). In contrast, Ku et al.'s sTPOR is actually part of the receptor and there is no suggestion or reason to believe that it can bind to an mpl receptor:

> "The current understanding of the mechanisms of soluble receptors include their role as inhibitors of their respective membrane bound receptors by competing for ligands, downregulators of the expression of membrane-bound receptors, stabilizing proteins of the ligands, and participants of ligand-induced signaling." (Ku et al., page 4129, right column).

If anything, the sTPOR utilized by Ku et al. would be expected to *counteract* any mpl ligand present in the system by binding it and making it unavailable to bind to the full membrane-bound TPO receptor and thus teaches away from applicants' invention.

The results of Ku et al. exemplify the uncertainty in the art of growing hematopoietic stem cells. Ku et al. clearly does not teach or suggest the use of an mpl ligand and a flt3 ligand for the stimulation of growth of hematopoietic stem cells and thus does not alone or in combination with any other cited reference make obvious applicants' presently claimed invention.

Kobayashi et al. does disclose experiments where a combination of SF, FL and TPO are examined for their ability to stimulate proliferation of $CD34^+$ cells. However, the results of Kobayashi et al. do not in any way teach or suggest that FL and TPO (or another mpl ligand) are useful in stimulating growth of pluripotent human hematopoietic stem cells without concomitant loss of pluripotency. First, it is not at all clear that Kobayashi et al. was using cells containing a subpopulation of pluripotent $CD34^+Thy\text{-}1^+Lin^-$ cells, as required by applicants' claimed methods. Kobayashi et al. used CD34+/c-Kit^{low}/$CD38^{neg/low}$ cells, which were isolated from human bone marrow. It is not known if these cells were also Thy-1^+. Even if they were, Kobayashi et al. demonstrates that a combination of SF, FL and TPO "supported formation of only few colonies from CD34+/c-Kit^{low}/$CD38^{neg/low}$ cells"

and that addition of IL-3 to that cytokine mix was necessary for a significant increase in the number of colonies (see Abstract). More importantly, Kobayashi et al. demonstrates that combinations of FL/TPO and SF/TPO in seven day suspension cultures stimulate the production of colony-forming cells which had lost their pluripotency ("Both FL and SF produced predominantly GM colony-forming cells in synergy with TPO" page 427, right column).

A fair reading of Kobayashi et al. actually teaches away from applicants' invention. That reference demonstrates that FL and TPO (optionally together with SF) does not effectively support colony formation without the addition of IL-3 and that in the presence of IL-3 the cells that do proliferate have lost their pluripotency. This is in direct contrast to applicants' invention which demonstrates that FL and TPO (optionally together with KL) are sufficient to stimulate proliferation of pluripotent human hematopoietic cells without the loss of pluripotency. Applicants' methods do not require the addition of IL-3 to human pluripotent hematopoietic cells at any point either prior to or after transducing the cells. The addition of IL-3 is optional (see claims 20, 25 and 40) and applicants were the first to discover that TPO can actually overcome the effect of IL-3 to promote differentiation (specification, page 31, lines 17-19).

Like Kobayashi et al., Ohmizomo et al. also demonstrates that combinations of TPO, FL and IL-3 cause expansion of committed progenitors in a $CD34^+$ population of cells (page 528, left column). This does not suggest, and in fact teaches away from, applicants' claimed invention -- the use of TPO and FL (with or without the additional cytokines claimed in applicants' invention) to grow and expand a population of pluripotent human hematopoietic stem cells without loss of pluripotency. Ohmizomo et al. concludes that "IL-3 is the pivotal cytokine for the expansion of human hematopoietic progenitor cells in the presence of SCF or FL" (page 528, right column) and that TPO synergizes with IL-3/FL or IL-3/SCF to expand *committed* progenitor cells (cells which have lost their pluripotency). As stated above, applicants' invention does not require the use of IL-3 in any of the presently claimed growth or transducing conditions. Accordingly, Ohmizomo et al., either alone or in combination with other references, cannot render applicants' invention unpatentable.

Ramsfjell et al. studied the effect of TPO in combination with KL + FL or KL +IL-3 +IL-6 on what they refer to as "multipotent" human progenitor cells. Ramsfjell

et al. does demonstrate that a combination of TPO/KL/FL causes an increase in proliferation and clonal growth of $CD34^+CD38^-$ cells and that many of those cells have myeloid/erythroid potential. Importantly, Ramsfjell et al. "could not determine whether the population recruited by Tpo also had a lymphoid potential", nor did they observe the production of megakaryocytes (p. 5176, left column). This led Ramsfjell et al. to state that "it is important to note that it has yet to be established whether Tpo (or any other known cytokine) might expand the true long term reconstituting pluripotent stem cell" (page 5176, right column). This is what distinguishes the present invention. Applicants have, for the first time, demonstrated that treatment of a human hematopoietic $CD34^+$ cell population containing a subpopulation of pluripotent $CD34^+Thy\text{-}1^+Lin^-$ cells can be maintained, proliferated and transformed in the presence of TPO and FL (optionally with additional specified cytokines) without a loss of pluripotency.

Ramsfjell et al.'s results do not demonstrate this. The conflicting results of Kobyashi et al. and Ohmizomo et al. -- combined with Ramsfjell et al.'s inability to demonstrate that TPO/KL/FL treatment did not destroy the lymphoid potential of the treated cells and doubts as to the true pluripotent nature of cells treated with that combination -- highlight the uncertainty in the field. The combination of these references falls far short of teaching applicants' claimed invention. They provide no guidance, nor expectation of success to one of skill in the art. At best, the combination of the conflicting and uncertain results of Ramsfjell et al., Ku et al., Kobyashi et al. and Ohmizomo et al. simply provides an invitation to experiment to resolve the conflict and find a suitable combination of cytokines in which one can maintain and grow human pluripotent hematopoietic stem cells without destroying their pluripotent ability. Furthermore, there can be no motivation to transduce such cells with a heterologous gene if one cannot envision the appropriate growth conditions. Accordingly, this combination of references is insufficient to support an obviousness rejection.

The additional references cited by the Examiner fail to provide any additional information that might allow one of skill in the art to reach applicants' invention. Szilvassy et al. does not use either FL or TPO alone or in combination. Fernandez et al. does not use TPO in combination with FL or any other cytokine. Moreover, Fernandez et al. utilizes committed dendritic cells and is not concerned with the pluripotent hematopoietic stem cells required by the amended claims. Tushinski et al.

and Fletcher et al. also do not teach or suggest a combination of FL and TPO. The same is true of Bodine et al., Murray et al., Nakahata et al., Hoffman et al., Fei et al. and Davis et al.

The Examiner's position with respect to all of the references referred to in the above paragraph is that each are relied upon "primarily to teach the use of different combinations of cytokines" (Office Action, p. 11). The Examiner repeatedly turns to the combination of Ramsfjell et al., Ku et al., Kobyashi et al. and Ohmizomo et al. for a teaching of the use of a combination of FL and TPO and FL/TPO/KL. However, as explained above, the combination of these four references does not teach or suggest what combination of cytokines would be useful for maintaining, growing and transducing pluripotent human hematopoietic stem cells *while maintaining their pluripotent properties*. Applicants were the first to teach this and therefore the claimed invention is patentable over the cited combination of references.

The discussion above and the cited references are mainly directed to cytokines that allow the maintenance and proliferation of pluripotent human hematopoietic cells without differentiation. Applicants' combination of cytokines together with the inclusion of fibronectin or a fibronectin analogue in the growth media and the subsequent transduction of those cells with a heterologous gene represent yet another patentable aspect of the present invention.

The Examiner cites Hanenberg et al. as teaching "enhanced retroviral delivery to HSCs with the use of fibronectin" (Office Action, page 14). However, Hanenberg et al. does not teach or suggest any synergistic effect between fibronectin and a cytokine mix containing FL and TPO on heterologous gene expression following transduction. The Examiner's attention is directed to Example 9 of the specification and to Figure 9. Here applicants compare the percentage of Lyt2-expressing cells in populations of human $CD34^+$ hematopoietic cells including a subpopulation of pluripotent $CD34^+Thy\text{-}1^+Lin^-$ hematopoietic stem cells following growth and transduction with the L Mily vector in different cytokine combinations ± a fibronectin analogue. The fibronectin analogue when combined with a cytokine combination that included FL and TPO, resulted in a 4-fold higher number of cells expressing Lyt-2 than in the absence of the analogue. This effect was not observed when the fibronectin analogue was added to cytokine combinations which lacked both FL and TPO. As far as applicants are aware, this is the first demonstration of successful transduction and subsequent expression of the transduced gene in human pluripotent

hematopoietic cells grown in the presence of fibronectin (or a fibronectin analogue) and a cytokine combination that includes FL and TPO.

Applicants' results demonstrate that the use of fibronectin or a fibronectin analogue in conjunction with FL and TPO (and optionally other cytokines specified in the claims) produces a surprising and unexpected synergistic effect on the expression of a heterologous gene transduced into a $CD34^+$ human hematopoietic cell population containing a subpopulation of pluripotent human hematopoietic stem cells. Moreover, following transduction of such cell population, expression is surprisingly enhanced in all $CD34^+$ cells, not just the pluripotent subpopulation (see Example 9 and Table 10). Such synergistic effect is not suggested, nor could be predicted, by Hanenberg et al., alone or in combination with any other cited art. As such, this aspect alone renders applicants' claimed invention patentable over the cited art.

Applicants' surprising and unexpected results combined with the uncertainty and contradictory results of the prior art with respect to identifying cytokine combinations capable of maintaining and proliferating human pluripotent hematopoietic cells while retaining pluripotency clearly show that the present invention is patentable. Applicants' presently claimed invention represents an inventive and non-obvious step over the prior art in a field where there was little if any predictability. Therefore, the Examiner should withdraw her §103 rejections.

Accordingly, applicants request that the Examiner enter the claim amendments presented herein, consider the foregoing remarks and allow the pending claims to pass to issue.

Respectfully submitted,

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